IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 4261
TRINH *et al.*	Art Unit: 1652
Appl. No. 09/306,986	Examiner: Hudson, R.
Filing date: May 7, 1999	Atty. Docket: 0942.4570001/RWE/FRC
For: **A Method for Synthesizing a Nucleic Acid Molecule Using a Ribonuclease**	

Brief on Appeal Under 37 C.F.R. § 41.37

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

A Notice of Appeal from the final rejection of claims 8-12, 56, and 70-73 was filed on September 30, 2005, and an Appeal Brief was filed on May 1, 2006. Prosecution was reopened on July 25, 2006 by the issuance of a non-final Office Action by the Examiner. Rather than filing a reply under 37 C.F.R. § 1.111, Appellants wish to reinstate the Appeal. *See* M.P.E.P. §§ 1204.01 and 1207.04. Accordingly, Appellants file herewith a second Notice of Appeal under 37 C.F.R. § 41.31 along with this complete new Appeal Brief under 37 C.F.R. § 41.37.

In accordance with M.P.E.P. § 1204.01, Appellants request that Notice of Appeal fee previously paid on September 30, 2005 ($500.00), and the Appeal Brief fee previously paid on May 1, 2006 ($500.00), be applied to the reinstated Appeal filed herewith.

It is not believed that extensions of time or fees are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional fees are due for additional extensions of time or fees set forth in 37 CFR 41.20 have been increased since they were previously paid and are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required are hereby authorized to be charged to our Deposit Account No. 50-3994.

Table of Contents

I. Real Party In Interest

The real party in interest in this appeal is Invitrogen Corporation.

II. Related Appeals and Interferences

No other prior or pending appeals, interferences or judicial proceedings are known to the Appellants, the Appellants' legal representative, or assignee which may be related to, or directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. Status of Claims

Claims 8-13, 56, and 70-75 are pending in the application.

Claims 1-7, 14-55, and 57-69 have been canceled.

Claims 8-12, 56 and 70-73 are rejected.

Claims 13, 74 and 75 are objected to.

IV. Status of Amendments

No amendments were filed subsequent to the final rejection.

V. Summary of Claimed Subject Matter

Claim 8 is the sole independent claim involved in this Appeal. The invention defined by claim 8 relates generally to methods of synthesizing polynucleotides in the presence of ribonucleases. (*See* specification at page 2, lines 24-26). More specifically, the currently claimed invention relates to methods of synthesizing a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA. The claimed methods comprise mixing the preparation of RNA and double-stranded DNA with one or more DNA polymerases and one or more peptides or polypeptides having ribonuclease activity. The peptides or polypeptides having ribonuclease activity are capable of degrading single-stranded RNA. The claimed methods further comprise incubating the mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of the double-stranded DNA and under which the peptides or polypeptides having ribonuclease activity degrade the single-stranded RNA.

Support for claim 8 can be found throughout the specification, for example, at page 3, lines 18-26 through page 4, lines 1-14; at page 11, lines 21-23; at page 12, lines 21-23; at page 13, lines 1-9 and 13-16; at page 15, lines 10-12; at page 15, lines 23 through page 16, lines 1-12; at page 18, line 5 through page 19, line 5; and in Example 1 at page 25, line 15 through page 27, line 13.

VI. Grounds of Rejection to be Reviewed on Appeal

There are two grounds of rejection to be reviewed on appeal:

Ground 1: Claims 8-12, 56 and 70-73 were rejected under 35 U.S.C. § 102(b) as being anticipated by Major, *Biotechniques 12*:40-43 (1992) (Exhibit 1), as evidenced by Deana and Belasco, *Mol. Microbiol. 51*:1205-1217 (2004) (Exhibit 5).

Ground 2: Claims 8-12, 56, 70, 71, and 73 stand rejected under 35 U.S.C. 103(a), as being unpatentable over Major, *Biotechniques 12*:40-43 (1992) (Exhibit 1) and Maudru *et al., J. Virological Methods 66*:247-261 (1997) (Exhibit 2). Appellants have traversed this rejection.

VII. Argument

A. Ground 1: Anticipation

1. Legal Standard for Anticipation

Under 35 USC § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984); *see also PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996) ("[t]o anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter.")

Inherent disclosures of a prior art reference may be relied upon in a rejection of claims under § 102. *See In re Napier*, 55 F.3d 610, 613, 34 USPQ2d 1782, 1784 (Fed. Cir. 1995) ("The inherent teaching of a prior art reference, a question of fact, arises both in the context of anticipation and obviousness.") *See also In re Grasselli*, 713 F.2d 731,

739, 218 USPQ 769, 775 (Fed. Cir. 1983). A rejection based on a theory of inherency, however, must be supported by "a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990).

2. The References Cited In Support of the Anticipation Rejection

(a) The Major Reference

The Major reference discloses a PCR-based assay for screening point mutations. The method of Major involves the use of oligonucleotide primers, the 3'-terminal nucleotide of which may or may not be complementary to a specific nucleotide in a target sequence. *See* Major at page 40, Figure 1. The principle behind this assay is that PCR amplification should occur only when the 3'-terminal nucleotide of a primer base-pairs with the corresponding nucleotide on the template nucleic acid, and should not occur when the 3'-terminal nucleotide of a primer does not base-pair with the corresponding nucleotide on the template nucleic acid. *See* Major at page 42, left column.

Counter to this expectation, Major observed in his assay that "Even bacterial colony lysates showed clear negative results with all three 3'-terminal mismatches; however T:T mismatches gave some extra minor bands." *See* Major at page 42, bottom center column. Thus, Major attributed the "extra minor bands" to amplification products produced from oligonucleotides that have 3'-terminal nucleotide mismatches. Major also noted that other researchers had observed PCR amplification despite the presence of a 3'-terminal nucleotide mismatch. *See* Major at page 42, center column (citing Wu *et al.*,

Proc. Natl. Acad. Sci. USA 86:2757-2760 (1989) and Kwok *et al.*, *Nucl. Acids Res. 18*:999-1005 (1990)).

(b) The Deana and Belasco Reference

The Deana and Belasco reference indicates that RNAse E is an *E. coli* ribonuclease and that RNase G is a homologue of RNase E. *See* Deana and Belasco, page 1205, Abstract. The Examiner has acknowledged that the Deana and Belasco reference is *not* prior art with respect to this application and is cited solely to support the Examiner's theory of inherent anticipation. *See* Office Action dated July 25, 2006, page 3.

3. The Examiner's Position With Respect to the Anticipation Rejection

The anticipation rejection is based on the Examiner's position that:

> Major teaches a method which comprises the synthesis of a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA, said method comprising mixing the preparation with one or more DNA polymerases and incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of a template nucleic acid molecule. The method taught by Major specifically involves PCR amplification, using Taq DNA polymerase, of a DNA fragment from the expression plasmid, pBluescript 11 SK(+), either sampled directly from JM109 E. coli colonies or from a bacterial plasmid isolate.

(Office Action dated July 25, 2006, page 3). The Examiner has not argued that the Major reference teaches the use of a ribonuclease in a nucleic acid synthesis reaction. Rather, the Examiner has simply stated that:

> The inclusion of ribonuclease in the mixture taught by Major is evidenced by Deana Belasco (Mol. Microbiology,

> Vol 51 No. 4, pp 1205-1217, 2004) who teach that *E. coli* inherently comprise a number of Rnases that are capable of degrading single stranded RNA.

(Office Action dated July 25, 2006, page 3). Thus, the anticipation rejection is based on a theory of inherent anticipation by the Major reference, and relies upon the Deana and Belasco reference as a supporting secondary reference.

4. The Appellants' Position With Respect to the Anticipation Rejection

The Major reference does not anticipate the currently pending claims. Major discloses PCR-based assays using either bacterial lysate or mini-prep DNA as starting material. *See* Major, page 42, top left column, and Fig. 2. Specifically, the PCR-based assays described in the Major reference contained:

> 10 mM Tris-HCl (pH 8.3 at 25°C), 50 mM KCl, 1.5 mM MgCl2, 0.01% (wt/vol) gelatin, 200 μM of each of the four deoxynucleotide triphosphates (dNTPs), 1 μM of each primer, 1-5 ng or mini-prep template DNA or 10 μl of *clarified bacterial colony lysate* as described, 0.25 units of Perfect Match and 1.25 units of *Taq* DNA polymerase.

See Major, page 40, top right column, emphasis added. The Examiner's position seems to be that the "clarified bacterial colony lysate" used in the Major reference inherently contains RNases. However, because a clarified bacterial colony lysate does not necessarily contain RNAases, the Deana and Belasco reference does not support the Examiners inherent anticipation argument.

Moreover, even if the Examiner's inherent anticipation argument is correct, there is nothing in the Major reference that teaches mixing a preparation comprising RNA and double-stranded DNA with one or more DNA polymerases and one or more peptides or polypeptides having ribonuclease activity, as specified by the currently pending claims.

The Major reference discloses the addition of a DNA polymerase to a sample (*i.e.*, clarified bacterial colony lysate), which may (or may not) contain an RNase. Adding a DNA polymerase to a biological sample that already contains an RNase, where the RNase is derived from the cellular source of the sample, is clearly distinct from *mixing* the sample with a DNA polymerase and an RNase, as required by the present claims. Thus, regardless of whether *E. coli* inherently contain RNases, the Major reference does not teach a method that includes all of the steps that are encompassed by the currently presented claims.

B. Ground 2: Obviousness

1. Legal Standard for Obviousness

In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. *See In re Piasecki*, 745 F.2d 1468, 1471-73, 223 USPQ 785, 788 (Fed. Cir. 1984). To meet this burden, the Examiner must satisfy three requirements. First, all of the claim limitations must be taught or suggest by the prior art. *See In re Royka*, 490 F.2d 981, 984-85, 180 USPQ 580, 583 (CCPA 1974); *see also In re Glaug*, 283 F.3d 1335, 1341-42, 62 USPQ2d 1151, 1154 (Fed. Cir. 2002) (finding a claim not obvious because the prior art did not teach "spaced zones of adhesive" as recited in the claim); *In re Rijckaert*, 9 F.3d 1531, 1533, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (finding a claim not obvious because the prior art did not teach all claim limitations). Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teaching. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453,

1457-58 (Fed. Cir. 1998). Third, there must be a reasonable expectation of success. *See In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir.1986). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants' disclosure. *See In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Evidence of a suggestion, teaching, or motivation to combine prior art references may flow, *inter alia*, from the references themselves, the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. *See In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999). Although a reference need not expressly teach that the disclosure contained therein should be combined with another, *see Motorola, Inc. v. Interdigital Tech. Corp.*, 121 F.3d 1461, 1472, 43 USPQ2d 1418, 1489 (Fed. Cir. 1997), the showing of combinability, in whatever form, must nevertheless be "clear and particular." *Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. "Board conclusory statements regarding the teaching of multiple references, standing alone, are not evidence." *Id.*, 175 F.3d at 999, 50 USPQ2d at 1617, *see also In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.")

2. The References Cited In Support of the Obviousness Rejection

(a) The Major Reference

The Major Reference is discussed in Section VII.A.2(a), above.

(b) The Maudru Reference

The Maudru reference discloses an assay for detecting the presence of retroviral reverse transcriptases in a sample. *See* Maudru, sentence bridging pages 247-248. The assay involves a reverse transcription step in which a sample suspected of containing a retroviral reverse transcriptase is combined with an RNA template. Any cDNA produced is then amplified by PCR using a DNA polymerase. *See* Maudru at page 248, left column. The principle behind this assay is that only samples that contain a retroviral reverse transcriptase should produce cDNA and consequently PCR-amplified DNA, while samples that do not contain a retroviral reverse transcriptase should produce no cDNA and consequently no PCR-amplified DNA.

Counter to this expectation, Maudru observed that "background" PCR amplification can occur even when cDNA is not produced from the reverse transcription step; as in control reactions that contain no retroviral reverse transcriptase. *See* Madru at page 256, left column. Maudru observed that this "background" could be eliminated by treating the sample with a ribonuclease to degrade the RNA template before adding the DNA polymerase for the PCR amplification step. *See* Maudru at page 258, left column. Thus, Maudru attributes the background amplification products to an intrinsic RNA-dependent DNA polymerase (*i.e.*, reverse transcriptase) activity of the AmpliTaq DNA polymerase used in the assay. *See* Maudru at page 256, left column.

3. The Examiner's Position With Respect to the Obviousness Rejection

The outstanding obviousness rejection is based on the Examiner's assertions that (1) a person of ordinary skill in the art would have attributed the "extra minor bands" mentioned in the Major reference to the presence of contaminating RNA in the reactions;

and (2) that a person of ordinary skill in the art would have been motivated to include a ribonuclease digestion step in the Major assay based on Maudru's teaching that "the background signal in a similar assay was found to be due to an intrinsic RNA-dependent DNA polymerase activity of the *Taq* DNA polymerase." Specifically, the Examiner has stated that:

> While Major does not attribute background difficulties to contaminating RNA, one of skill in the art would realize that given the employment of the method of Major to bacterial lysates, there would be a substantial amount of background RNA in the preparation. This knowledge in combination with that taught by Maudru et al. stating that the background signal in a similar assay was found to be due to an intrinsic RNA-dependent DNA polymerase activity of the *Taq* DNA polymerase would lead one of skill in the art who was attempting to successfully use a PCR method to screen for small mutations to include a ribonuclease digestion step prior to PCR amplification as a means of making the assay more sensitive. In support of the above, applicants attention is drawn to Major, page 42, middle column, which states "the present results indicate that all three possible terminal T mismatches can be equally discriminated under standard PCR conditions, especially when using mini-prep DNA". Such a statement clearly supports that even Major recognized the taught method had different results or sensitivities depending on the template used (noting the reference to "especially"), although Major did not comment on the specific difference of the two different types of template preparations. One of skill in the art would understand that the difference was likely the presence of contaminating material, such as RNA.

See Office Action dated March 17, 2004 at pages 8-9 (emphasis in original).

4. The Appellants' Position With Respect to the Obviousness Rejection

Claims 8-12, 56, 70, 71 and 73 are not obvious over the Major and Maudru references.

(a) The Examiner's Argument That a Person of Ordinary Skill in the Art Would Have Attributed the "Extra Minor Bands" Mentioned in the Major Reference to the Presence of Contaminating RNA in the Reactions is Incorrect

As discussed above, Major himself did not attribute the "extra minor bands" that he observed in his assay to contaminating RNA. Rather, he attributed the "extra minor bands" to amplification products produced from oligonucleotides that have 3'-terminal nucleotide mismatches. *See* Major at page 42, bottom center column. Major's attribution is consistent with the observations of other researchers who he noted had observed PCR amplification despite the presence of a 3'-terminal nucleotide mismatch. *See* Major at page 42, center column (citing Wu *et al.*, *Proc. Natl. Acad. Sci. USA 86*:2757-2760 (1989) and Kwok *et al.*, *Nucl. Acids Res. 18*:999-1005 (1990)).

Despite the fact that Major himself, in a manner consistent with the observations with others, attributed the "extra minor bands" to something other than contaminating RNA, the Examiner's position is: (1) that a person of ordinary skill in the art would have recognized that bacterial colony lysates contain a substantial amount of RNA, (2) that Major indicates that better discrimination of terminal T mismatches was obtained using mini-prep DNA than with bacterial colony lysates; and therefore that (3) a person of ordinary skill in the art would have attributed the difference in terminal T mismatch discrimination to the presence of RNA in bacterial colony lysates.

The Examiner's reasoning is flawed and cannot support a *prima facie* case of obviousness. First, persons of ordinary skill in the art would have appreciated at the time of the effective filing date of the present application that bacterial colony lysates contain many factors other than RNA (*e.g.*, proteins, salts, lipids, signaling molecules, etc.), and

that, like RNA, such factors are absent from mini-prep DNA. The Examiner has failed to explain why a person of ordinary skill in the art, considering all the factors found in bacterial colony lysates, would have specifically regarded RNA as the one factor responsible the difference in 3'-terminal T mismatch discrimination described in Major.

The Examiner's implication that a person of ordinary skill in the art would have believed that errors in terminal nucleotide mismatch discrimination occur only in bacterial colony lysates (which contain RNA) but not in other DNA preparations (which do not contain RNA) is incorrect. To the contrary, it was well known in the art at the time of the effective filing date of the present application that errors in terminal mismatch discrimination (*i.e.*, extension of oligonucleotides having mismatched 3'-terminal nucleotides) can and do occur in purified DNA preparations lacking RNA.

For example, Kwok *et al.*, *Nucl. Acids Res. 18*:999-1005 (1990) ("Kwok") (Exhibit 3), describe assay systems involving the use of plasmid DNA or PCR-generated products as templates for PCR extension reactions. *See* Kwok, page 1001, left column. Using these samples – *which lacked RNA* – template amplification was observed using several primers having mismatched 3'-terminal nucleotides. *See, e.g.*, Kwok at page 1001, right column ("the presence of a T at the 3' end of the primer provided efficient amplification irrespective of the corresponding nucleotide in the template.") Kwok attributed these errors in terminal mismatch discrimination to factors such as dNTP concentration and primer length. *See* Kwok at page 1004, right column. (Kwok was cited in Major as reference No. 6.) Clearly, RNA could not have been a factor for the terminal mismatch discrimination errors described in Kwok.

Because it was well known in the art at the time of the effective filing date of the present application that errors in terminal mismatch discrimination occurred in samples that did not contain any RNA, a person of ordinary skill in the art would have appreciated that factors other than RNA were responsible for errors in terminal mismatch discrimination. A discussion of other factors reported to influence 3'-terminal mismatch discrimination is found in Charlieu, "Chapter 12, Distinction Between Almost-Identical DNA Sequences by Polymerase Chain Reaction," in PCR Technology Current Innovations, pp. 101-106, Griffin and Griffin Eds., (1994) (Exhibit 4). Factors mentioned in Charlieu include the nature of the DNA template, the nucleotide concentration, $MgCl_2$ concentration, *Taq* DNA polymerase concentration, and the presence of chemicals such as tetramethylammonium chloride (TMAC) or Perfect Match. *See* Charlieu at pages 105-106. Charlieu concludes that "[t]he stringency of PCR is defined by a combination of these factors." *See* Charlieu at page 106. Nowhere is it suggested that RNA can influence 3'-terminal mismatch discrimination. Thus, a person of ordinary skill in the art would not have regarded the difference in terminal mismatch discrimination alluded to in Major as being caused by RNA in bacterial colony lysates.

The Examiner has presented no evidence whatsoever to indicate that, at the time of the effective filing date of the present application, persons of ordinary skill in the art would have regarded RNA as a factor which might interfere with nucleic acid synthesis reactions. In fact, Applicants note that the only document of record that indicates that RNA can interfere with nucleic acid synthesis is *Applicants' own specification*. *See, e.g.*, specification at page 2, lines 20-23. Applicants' own specification, however, cannot be

used to establish a *prima facie* case of obviousness. *See In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Examiner has not pointed to any evidence to support the contention that, despite the large number and variety of factors that are found in bacterial colony lysates and not in mini-prep DNA, a person of ordinary skill in the art would have believed that RNA was the specific factor which caused the difference in terminal T mismatch discrimination mentioned in Major. Since this contention is the basis of the obviousness rejection and there is no evidence to support it, a *prima facie* case of obviousness has not been established.

(b) The Examiner's Argument That a Person of Ordinary Skill in the Art Would Have Been Motivated to Include a Ribonuclease Digestion Step in the Major Assay Based on Maudru's Teaching that "The Background Signal in a Similar Assay Was Found to be Due to an Intrinsic RNA-Dependent DNA Polymerase Activity of the Taq DNA Polymerase" is Incorrect

The Major and Maudru references relate to very different, non-analogous assay systems, and persons of ordinary skill in the art would have had no motivation to associate or combine their teachings. Furthermore, Maudru attributes the presence of background amplification products in an assay designed to detect reverse transcriptases to an intrinsic RNA-dependent DNA polymerase activity of the DNA polymerase used in the assay. These background amplification products arise for a completely different reason than the "extra minor bands" mentioned in Major, which were caused by the extension of primers having a 3'-terminal mismatch. Because the background amplification products in Maudru arise for an entirely different reason than the "extra minor bands" observed in the Major assay, persons of ordinary skill in the art would not

have been motivated to apply Maudru's strategy to reduce background amplification to address the shortcoming of the Major's assay. That is, persons of ordinary skill in the art would not have been motivated to use a strategy shown to be effective to eliminate background amplification caused by an inherent RNA-dependent DNA polymerase activity to address the presence of "extra minor bands" caused by the extension of primers having 3'-terminal mismatches observed in the Major's assays. Persons of ordinary skill in the art would not have attributed the "extra minor bands" caused by the extension of primers having 3'-terminal mismatches to an RNA-dependent DNA polymerase activity of *Taq* polymerase, especially since (as discussed above) such extension was known to occur in systems that lack RNA. Thus, a person of ordinary skill in the art would not have been motivated to combine the ribonuclease treatment step of Maudru with the assay of Major.

C. Conclusion

In view of the forgoing discussion, Appellants respectfully submit that the subject matter defined by claims 8-12, 56, 70, 71 and 73 is novel and non-obvious over the cited art and that the Examiner has not met the burden of establishing a *prima facie* case of anticipation or obviousness. Accordingly, Appellants respectfully request that the Board reverses the Examiner's rejections of these claims under 35 U.S.C. §§ 102 and 103 and remand this application for issue.

Respectfully submitted,

/Natalie A. Davis/

Natalie A. Davis
Agent for Applicants
Reg. No. 53,849
Ph: 760-268-7469

Date: October 24, 2006

VIII. Claims Appendix

8. A method for synthesizing a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA, said method comprising:

a) mixing the preparation with one or more DNA polymerases, and one or more peptides or polypeptides having ribonuclease activity, wherein said peptides or polypeptides having ribonuclease activity are capable of degrading single-stranded RNA; and

b) incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said double-stranded DNA and under which said peptides or polypeptides having ribonuclease activity degrade said single-stranded RNA.

9. The method according to claim 8, wherein said peptide or polypeptide having ribonuclease activity is selected from the group consisting of: RNase A, RNase T1, RNase S, RNase B, RNase C, RNase T2 and enzymatically active fragments, variants, derivatives or mutants thereof.

10. The method according to claim 8, wherein said mixture further comprises one or more components selected from the group consisting of: a) at least one nucleotide; b) at least one suitable buffer for nucleic acid synthesis; and c) at least one primer.

11. The method according to claim 8, wherein said DNA polymerase is thermostable.

12. The method according to claim 11, wherein said thermostable DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tne DNA polymerase, Tma DNA polymerase, Tth DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, Pyrococcus species GB-D DNA polymerase, Pwo DNA polymerase, Bst DNA polymerase, Bca DNA polymerase, Tfl DNA polymerase and enzymatically active fragments, variants, derivatives or mutants thereof.

13. The method according to claim 10, wherein one or more of said nucleotides are detectably labeled.

56. The method of claim 8, wherein said preparation is from any cell or tissue selected from the group consisting of bacteria; insect; bird; fish; plant; yeast; prokaryote; eukaryote; and mammals.

70. A method according to claim 8, wherein said double-stranded DNA comprises an expression vector.

71. A method according to claim 8, wherein said double-stranded DNA comprises a cloning vector.

72. A method according to claim 8, wherein said double-stranded DNA comprises genomic DNA.

73. A method according to claim 8, wherein said double-stranded DNA comprises a plasmid or a cosmid.

74. A method according to claim 8, wherein said double-stranded DNA comprises viral DNA.

75. A method according to claim 8, wherein said double-stranded DNA comprises phage DNA.

IX. Evidence Appendix

Exhibit	Title of Exhibit	Location in Record
Exhibit 1	Major, *Biotechniques 12*:40-43 (1992)	Cited by Examiner in Office Action dated February 11, 2003
Exhibit 2	Maudru *et al.*, *J. Virological Methods 66*:247-261 (1997)	Cited by Examiner in Office Action dated January 2, 2001
Exhibit 3	Kwok *et al.*, *Nucl. Acids Res. 18*:999-1005 (1990)	Submitted by Applicants with Amendment and Reply Filed on June 17, 2004, entry of which was directed by way of Request for Continued Examination, filed on September 8, 2004
Exhibit 4	Charlieu, Ch. 12 in *PCR Technology Current Innovations*, pp. 101-106 (1994	Submitted by Applicants with Amendment and Reply Filed on June 17, 2004, entry of which was directed by way of Request for Continued Examination, filed on September 8, 2004
Exhibit 5	Deana and Belasco, *Mol. Microbiol. 51*:1205-1217 (2004)	Cited by Examiner in Office Action dated July 25, 2006

X. Related Proceedings Appendix

None.